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BRANCHING IN THE OPHIOGLOSSACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 202

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(WITH PLATES XX AND XXI AND SIX FIGURES)

The occurrence of branching of the rhizome in this family was mentioned by ROEPER (13) in 1859, when he described and figured specimens of *Botrychium Lunaria* in which the rhizome bore lateral branches. The origin of such branches was investigated by BRUCHMANN (2), who concluded that they were from adventitious buds of superficial origin. FARMER and FREEMAN (4) had already ascribed the occasional monopodial branching of *Helminthostachys* to the occurrence of adventitious buds. In 1902, GWYNNE-VAUGHAN (5) pointed out that small conical masses of parenchyma occur regularly in the axils of the leaves in this genus and suggested that these are vestigial buds. This view has been confirmed by LANG (8), who found that the branches are always axillary in position. The same writer (9) has also shown the regular occurrence of similar vestigial buds in the axils of the leaves in *B. Lunaria*, and has demonstrated that the branches of the rhizome arise from these, and not from buds of adventitious origin, as stated by BRUCHMANN.

A branching rhizome of *Ophioglossum vulgatum* was figured by STENZEL (14) in 1858; although no statement was made, the figure clearly indicates that the branching is dichotomous. VAN TIEGHEM (15) reported similar specimens and, disregarding ROEPER'S figures, stated that all branching of the rhizome in this family is

dichotomous. This view of the character of the branching was confirmed for *O. vulgatum* by POIRAULT (11).

The occurrence of the two methods of branching within this family has suggested the desirability of further examination of this feature. This investigation has accordingly been undertaken with a view to securing further data bearing upon the relation of the three genera to each other and of the family to other Pteridophytes.

Ophioglossum

POIRAULT (11) made the first critical examination of the nature of the branching of *O. vulgatum*. He concluded that the branching is usually only apparent, and that it is due to the development of a stem bud upon a young root before the root has broken through the



FIG. 1.—Transverse sections of a branching rhizome of *Ophioglossum vulgatum*; only the xylem is shown; $\times 7$.

cortex of the parent rhizome. In a single instance true branching was found, and anatomical examination showed this to be dichotomy. So far as the writer is aware, there is no record of branched rhizomes in other species of this genus.

Ophioglossum vulgatum.—In the examination of some 300 specimens of this species, five branched rhizomes were found. These were examined in serial sections, and in all cases the original stele has divided into two equal and similar steles (fig. 1). There can be no doubt that this is dichotomy, as contrasted with monopodial branching. The term “dichotomy” is here used to denote that branching in which a stem divides into two equal stems, as in *Lycopodium*, and is not meant to imply an exactly equal division of the apical cell of the rhizome.

Ophioglossum pendulum.—In the examination of some 100 rhizomes of this species, two branched specimens were found. As

in *O. vulgatum*, the branching is dichotomous, and the two branches in each case showed an almost exact equality.

In the examination of the material of these two species of *Ophioglossum*, careful search was made for evidence of axillary buds, such as have been described for *Helminthostachys* by GWYNNE-VAUGHAN (5), and for *Botrychium Lunaria* by LANG (9). No such structures have been found in any case; neither is there any evidence of adventitious budding except upon roots. From this it may be concluded that the true branching of these species is always dichotomous.

Helminthostachys

LANG (8) has briefly described the anatomy of two branching rhizomes of this genus. In both, the branches are definitely axillary in position, and it is certain that they have developed from the vestigial buds described by GWYNNE-VAUGHAN (5). In each case, the vascular supply of the branch comes from a mass of accessory xylem which develops outside the usual xylem of the stele, either locally at the base of the branch, or surrounding the stele. There is no connection between the branch stele and the subtending leaf trace.¹

Material of *Helminthostachys* has not been available during the progress of this investigation.

Botrychium

THE OCCURRENCE OF AXILLARY BUDS

All the investigation of branching and of the occurrence of buds has been confined to examination of *B. Lunaria*. On this account, it has seemed advisable to examine other species of this genus with regard to these points. Rhizomes of five species have been secured with branching specimens representing four of these species.

The genus *Botrychium*, as organized by PRANTL (12), consists of two sections: EUBOTRYCHIUM, with five species; and PHYLLOTRICHIMUM, with eleven species. The latter section is divided into

¹ The full description of this material has just been published (LANG, WM. H., Studies in the morphology and anatomy of the Ophioglossaceae. III. On the anatomy and branching of the rhizome of *Helminthostachys zeylanica*. Ann. Botany 29:1-54. pls. 1-3. figs. 1-8. 1915). In this paper LANG concludes that although no regular cambium is present, "the development of this accessory xylem should rightly come under the conception of secondary thickening."

two subsections: TERNATA, of eight species; and CICUTARIA, of three species. The material investigated consists of rhizomes of *B. ramosum* and *B. lanceolatum* var. *angustisegmentum*, of the section EUBOTRYCHIUM, to which *B. Lunaria* also belongs; of *B. obliquum* and *B. ternatum* var. *intermedium*, of the subsection TERNATA; and of *B. virginianum*, of the subsection CICUTARIA.

Rhizomes of these five species were examined in serial sections for the presence of such axillary buds as have been described by

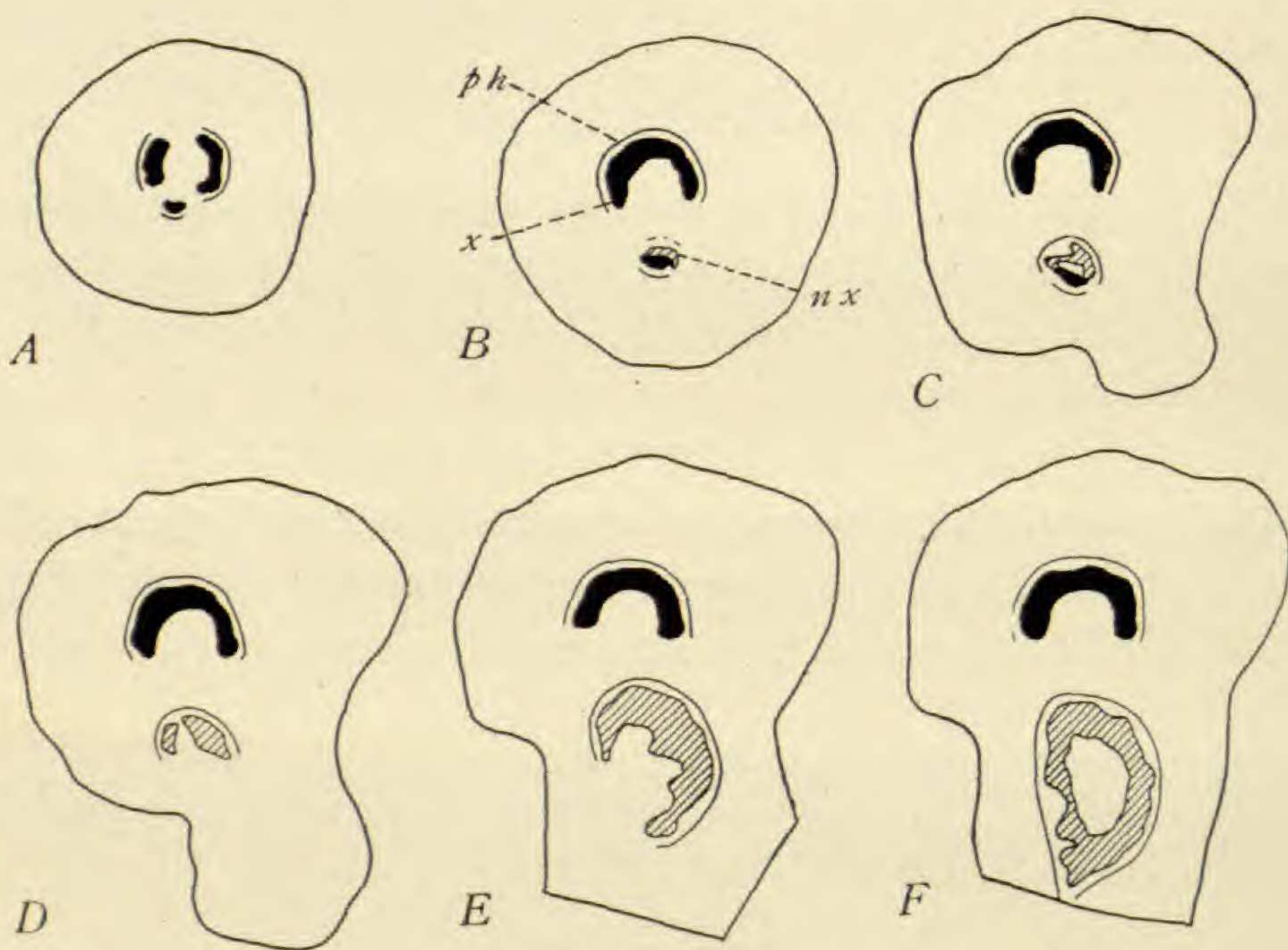


FIG. 2.—Transverse sections of a branching rhizome of *Botrychium lanceolatum* var. *angustisegmentum*: *x*, original xylem of the stem and leaf trace; *nx*, xylem formed after the injury; *ph*, phloem; $\times 12$.

LANG. In all these species, such buds are uniformly present in rhizomes of all ages, and it may be assumed that they are of constant occurrence in all species of the genus. Their origin and development will be considered later.

VASCULAR CONNECTIONS OF THE BRANCHES

ROEPER'S (13) figures represent rhizomes of *B. Lunaria* with 2-7 lateral branches. HOLLE (6) figured a single branching specimen of the same species in longitudinal section, and pointed out that in this case the branch stele connects with the trace of the leaf

immediately below. LANG (9) has given in detail the vascular connections of the branches in five specimens of this species; he concluded that "the chief vascular supply of the branch is derived from a development of xylem adaxially to the subtending leaf trace." This adaxial xylem is considered to be an extension of the margins of the leaf trace, and therefore centrifugal in character.

Botrychium lanceolatum var. *angustisegmentum*.—A single branching specimen of this species was secured. The terminal bud has been destroyed and the branch has arisen at a considerable distance below. As shown by fig. 2, the vascular system of the branch connects with the subtending leaf trace. After the trace has been separated from the stem stele for a considerable distance, tracheids appear on the adaxial side of the protoxylem of the trace; as shown by fig. 3, these are definitely centripetal in origin and occur in contact with the protoxylem. The mass of xylem formed in this way gives rise to the branch stele, which soon assumes the characters of the main stele. This differs from the general condition in *B. Lunaria*, as described by LANG, in that the branch connection arises from centripetal xylem of the leaf trace rather than from adaxial extension of the centrifugal xylem.

Botrychium ramosum.—Two rhizomes of this species, each bearing a single branch, were secured. In each case the terminal bud has been destroyed and the branch has developed at a considerable distance below. The vascular connections of the branches differ greatly from that described above.

Fig. 4 shows the vascular supply of the branch in the first of these specimens. As the leaf trace swings away from the stem stele, wings of primary and secondary xylem develop in the angle

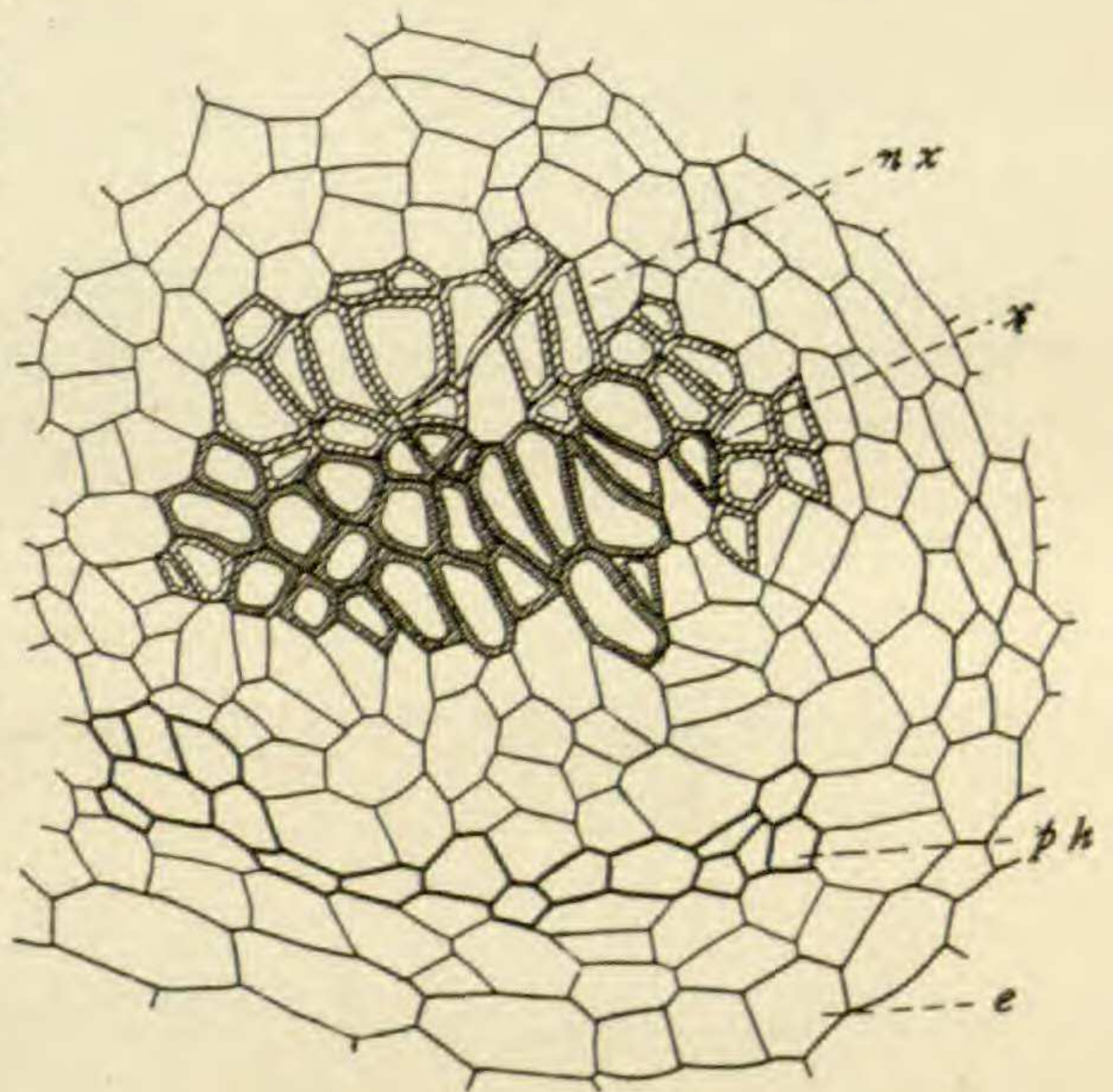


FIG. 3.—Detail of leaf trace of fig. 2, B: e, endodermis; $\times 157$.

between the two (figs. 4, *B*, and 7). At first these extend the entire distance between the two and give the appearance of expansion of the stele in the plane of the leaf trace; but later they separate from the stem stele, as shown by fig. 4, *C*. These wings may be considered to be adaxial extensions of the centrifugal xylem of the leaf trace; there is at no point any evidence of centripetal xylem. Soon after the separation from the stem stele, the leaf trace disappears, having been cut off by the absciss layer and carried out by periderm formation. This leaves two distinct wings of xylem and *each* of these

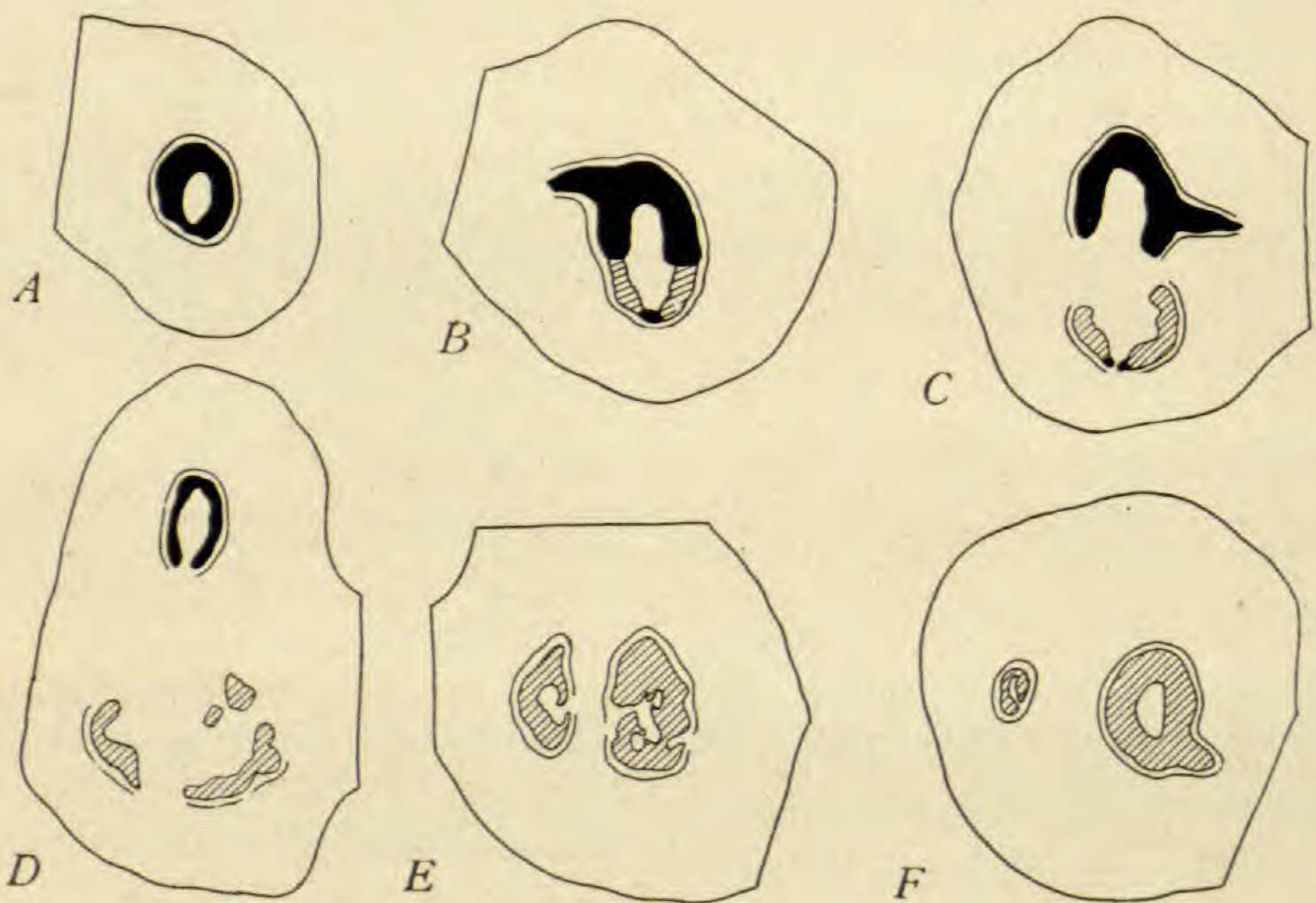


FIG. 4.—Transverse sections of a branching rhizome of *Botrychium ramosum*; $\times 12$.

by extension of its margins rounds up into a definite branch stele, as shown in figs. 4, *D*, *E*, *F*, and 8. One of these branch steles soon disappears, having apparently been unable to meet the competition; its apical region has been entirely obliterated by periderm formation. There can be little doubt that both these branch steles developed from a single axillary bud.

In the other branching specimen of this species, a wing of xylem extends between the leaf trace and the stem stele on one side only. It separates from the stem stele and at a slightly higher point the leaf trace disappears. By extension of its margins, the

wing of xylem rounds up to form a single branch stele. This is exactly the behavior of each of the two wings in the first specimen.

The vascular supply of the branch is here derived in part, at least, from adaxial extension of the centrifugal xylem of the leaf trace, as in *B. Lunaria*; but the formation of either one or two wings of xylem and the consequent formation of one or two branch steles is unique. The significance of this will be discussed later.

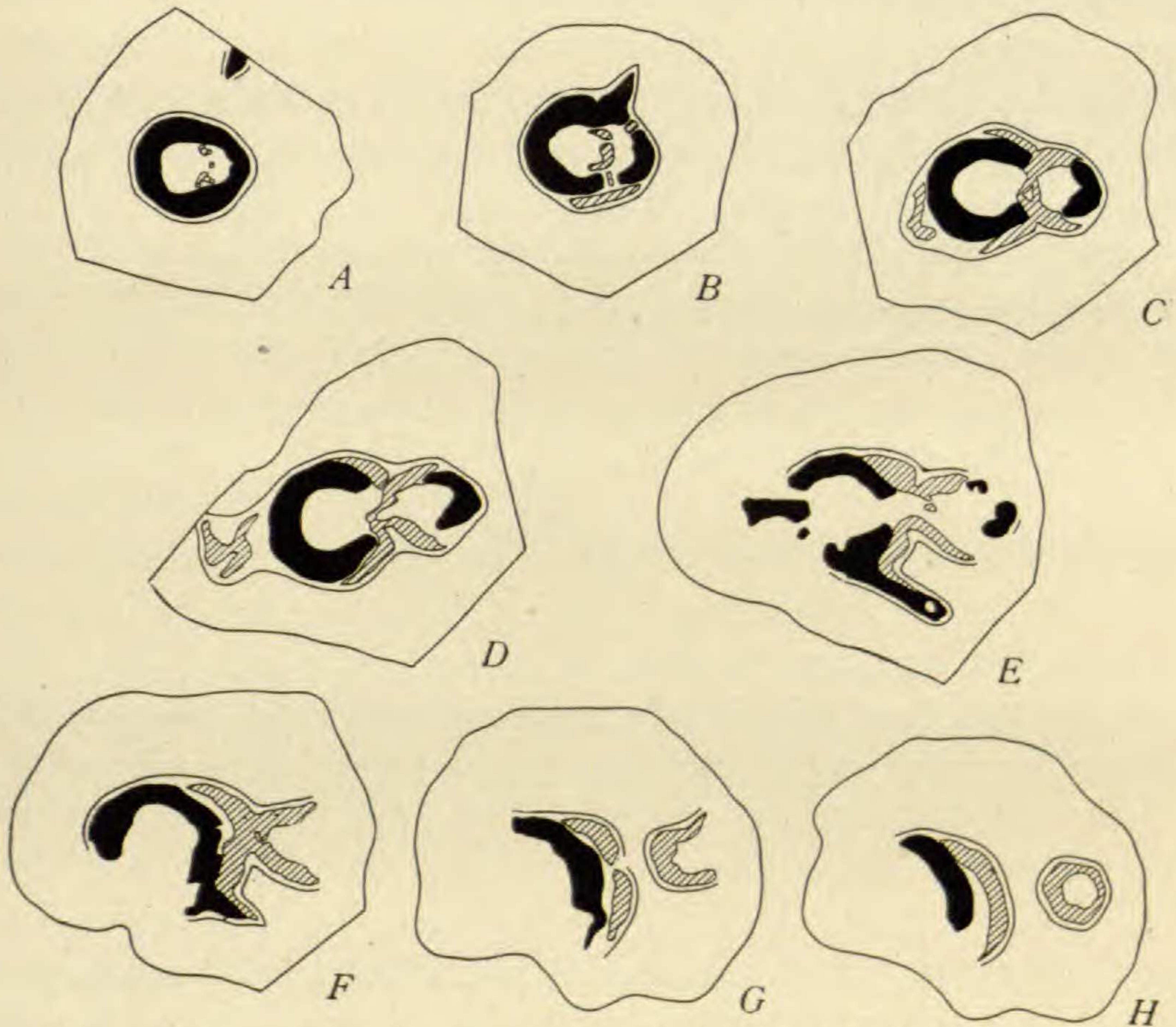


FIG. 5.—Transverse sections of a branching rhizome of *Botrychium virginianum*; X7.

Botrychium virginianum.—Two branching rhizomes of this species were examined; one of these bore two branches, the other a single one. Fig. 5 shows the vascular connections of the branch in one of these; the injury occurred at the side of the stem and destroyed almost the entire stele (fig. 5, G, H). As indicated by the figure, there is a development of xylem in the space between the outgoing leaf trace and the stem stele, as in *B. ramosum*; but

here both wings contribute to the formation of a single branch stele. Also procambium strands arise in the pith of the stem opposite the leaf trace (fig. 5, *A, B*); these develop a cambium directed toward the center of the stem, which produces a small amount of centripetal secondary wood which also contributes to the vascular supply of the branch. But the greater part of the vascular supply is furnished by the extra or accessory secondary xylem produced by the renewed activity of the cambium (fig. 5, *C*). In this case, therefore, the branch connection consists of (1) accessory secondary xylem, (2) adaxial extensions of the centrifugal xylem of the leaf trace, and (3) a small mass of centripetal secondary xylem originating within the pith of the leaf gap.

One of the other branches shows almost exactly the same connection, but the third branch shows a somewhat different situation. In it the vascular supply is composed principally of accessory secondary xylem; but a small mass of xylem appears in the pericycle of the stem and contributes to the supply of the branch. This pericyclic xylem is added to by a cambium directed toward the phloem; hence we may speak of primary and secondary pericyclic xylem, using the terms "primary" and "secondary" only to indicate the presence or absence of a definite cambium. The vascular supply of this branch, therefore, consists of (1) accessory secondary xylem and (2) primary and secondary pericyclic xylem.

Botrychium obliquum.—Four branching rhizomes of this species were secured; three of these bore two branches each, and the other had three branches. In all but one of these, the apical region had been destroyed; but in this one case the rhizome had been injured at the side below the apical region, but without destroying the entire stele. In all the other cases, the branches developed near the apical region. The vascular connection of the lowest of the three branches mentioned above is shown by fig. 6.

As shown by fig. 6, *A*, a very large development of accessory secondary xylem occurs entirely around the stele before the leaf trace separates; at the same level, a cambium has formed within the pith opposite the leaf trace and has developed a considerable mass of centripetal secondary xylem. The injury which occurred at the side of the stele at a slightly higher level has resulted in the

destruction of the greater portion of the original xylem of the stem; the attendant periderm formation has produced a considerable distortion of the stele. As the leaf trace separates from the stele (fig. 6, C) it is surrounded by a ring of xylem composed on the outer side of accessory secondary wood and on the inner side of centripetal secondary wood. This ring splits into two masses (fig. 6, D), and the leaf trace is cut off and carried out by periderm (fig. 6, D), and the leaf trace is cut off and carried out by periderm

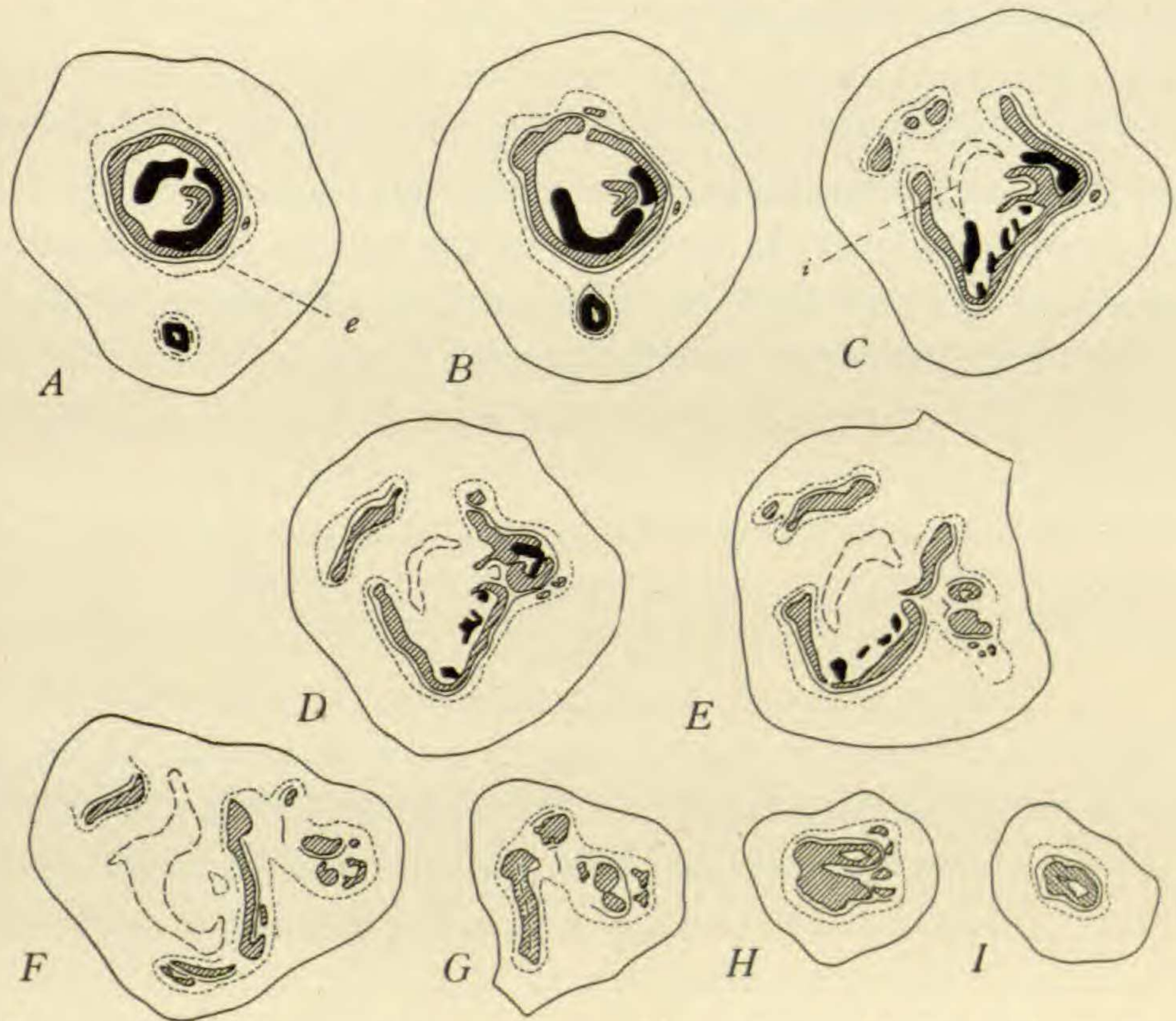


FIG. 6.—Transverse sections of a branching rhizome of *Botrychium obliquum*: *i*, injured region; $\times 3.5$.

formation. At a somewhat higher level, the accessory secondary xylem closes across the gap (fig. 6, F).

At a lower level (fig. 6, E), masses of xylem arise in the pericycle of one of the strands which formed part of the ring surrounding the leaf trace. Similar masses of pericyclic xylem occur outside the accessory secondary wood which closed the leaf gap (fig. 6, F). The development at this point results in the formation of a closed ring of xylem with cambium and phloem on the inside (fig. 6, G); at a slightly higher level, this becomes a tangled mass of tracheids

with occasional scattered sieve tubes and parenchyma cells. All these strands—the two produced by the splitting of the ring of xylem about the leaf trace, the strands which arise in the pericycle of one of those, and the tangled mass which is both accessory and pericyclic in origin—fuse to form the branch stele (fig. 6, *H*). Hence the vascular supply of the branch consists of (1) centripetal secondary xylem, (2) accessory secondary xylem, and (3) primary and secondary pericyclic xylem.

In other cases, numerous scattered tracheids appear in the pith below the point of separation of the leaf trace (fig. 9). As the trace leaves the stele, some of these swing out with it and contribute to the vascular supply of the branch. In such cases, there is not the slightest evidence of an internal cambium. Primary centripetal xylem of this kind occurs in the vascular supply of five of the nine branches, while abundant centripetal secondary wood is present in the other four.

It is not necessary to describe in detail the vascular connections of the other branches. Accessory secondary wood usually forms a considerable part of the supply and is present in every case. In six of the nine branches, wings of xylem form between the leaf and the trace and the stele, as in *B. ramosum*; in the other three such formations are entirely lacking. Xylem of pericyclic origin makes up a part of the connection in five cases; in three of these a definite cambium is present. The accessory secondary wood is the only formation which occurs in all the nine cases of this species.

The significance of the wide variation in the vascular supply of the branches will be discussed later.

WOUND REACTIONS

JEFFREY (7) has put forward the view that traumatic reactions are apt to be reversionary in character. BOWER (1) and LANG (9) have used this view in their contention that the pith of the Ophioglossaceae is stelar in character. In the examination of branching specimens of *Botrychium*, especially of *B. obliquum*, some further data upon the nature of the wound reactions of this genus have been secured.

Periderm formation.—The formation of cork at the point of injury has commonly been accepted as a direct response to conditions. In several of the rhizomes of *B. obliquum*, injuries affected all the tissues of the stem. In these cases, periderm is formed by every tissue that is still capable of growth; that is, by cortex, endodermis, pericycle, cambium, and pith. Periderm formation by the pith is shown in fig. 10.

The formation of vascular tissue by the pith.—In three specimens of *B. obliquum*, tracheids scattered through the pith were found in considerable numbers; fig. 9 shows a section through such a stem. In these cases the injury is at some distance above the point of appearance of the pith tracheids.

In both branching specimens of *B. virginianum* and in three of the four of *B. obliquum*, a considerable development of secondary xylem occurs within the pith; figs. 10 and 11 show examples of this. In all cases this development of centripetal secondary xylem occurs below and opposite the point of departure of a leaf trace. The cambium is always directed toward the center of the stele; well developed sieve tubes are present in most cases.

The formation of vascular tissues by the cambium.—In every injured rhizome of *B. virginianum* and *B. obliquum* a renewed activity of the cambium has occurred. Lignification does not always take place in the first elements produced by this renewed activity; this produces a narrow strip of cells of rectangular cross-section just outside the original xylem of the stem. Usually, however, tracheids resembling those of the original growth are produced by this renewed cambial activity (fig. 10); these make up the accessory secondary xylem mentioned above. It is to be noted that no such accessory xylem was found in either of the two species of the section EUBOTRYCHIUM that were examined, but that such formation occurs in *B. Lunaria* as reported by LANG.

The formation of vascular tissues by the pericycle.—In every injured rhizome of *B. virginianum* and *B. obliquum* the pericycle has produced xylem in greater or less amount. As already stated, these masses of pericyclic xylem sometimes contribute to the vascular supply of the branches. When the amount of xylem that occurs is relatively small, only a tangled mass of tracheids is formed; but

frequently a cambium develops and produces tracheids in definite radial rows (fig. 12). The cambium usually occurs on the inner side of the xylem, but in a few cases it is located on the outer side, and sieve tubes occur between it and the endodermis.

The endodermis.—A well marked external endodermis is constantly present in all species of *Botrychium* that were examined. The suberized band on the radial walls is particularly heavy in *B. virginianum* and *B. obliquum*. In uninjured rhizomes of these two species, the external endodermis is continuous except at the points of departure of leaf traces. As a trace swings out from the stele, the endodermis breaks at the side of the trace and closes again rather higher up across the leaf gap; the endodermis on the abaxial side of the trace persists for a short distance only. In injured rhizomes of these two species, the irregular growth of the various stelar tissues has produced distortions and breaks of the endodermal layer (fig. 6). In these specimens, the cells of the endodermis frequently divide by periclinal walls; but there is no evidence that vascular elements are ever formed as a result of this growth. In no case were vascular elements found definitely outside the external endodermis. The only internal endodermis found was that occasionally formed by the folding in of the margins of the external endodermis in cases of greatly disturbed steles (fig. 6, C).

Discussion.—The occurrence of scattered tracheids in the pith has been observed in *B. ternatum* by BOWER (1) and in *B. Lunaria* by LANG (9). As stated above, both scattered tracheids and distinct secondary xylem occur frequently in the pith of injured rhizomes of *B. virginianum* and *B. obliquum*. The occurrence of well organized strands of xylem in the pith of a rhizome of *Ophioglossum pendulum* has been reported by the writer (10). In view of these facts, it may be concluded that the pith is definitely stelar in character in *Botrychium* and probably in all three genera of the family.

A slight intrusion of cortical tissue might occur at the point of the break of the endodermis without affecting the pith; such an intrusion would affect only the tissues between the leaf trace and the leaf gap. It may be pointed out that in branching specimens of *B. ramosum*, *B. virginianum*, and *B. obliquum*, xylem is produced

in quantity at this location; hence this tissue is not an intrusion of the cortex, but is stelar in character. It is to be noted that this tissue is opposite a break in the endodermis, but not outside it.

The view is held that there is a morphological distinction between stelar and cortical tissues; that the suberization of the radial walls of a layer of cells is a physiological phenomenon which under the usual conditions of development occurs in the layer of cells next outside the stele; and that this suberization, under the usual conditions, may be considered an indication of the morphological boundary of the stele, but that it is subject to variation with physiological conditions. LANG has concluded that the internal endodermis of *B. Lunaria* is of physiological significance only, and has suggested that its development is associated with the long leaf gaps of the intermediate region of the rhizome. It is to be noted that the apparent internal endodermis in injured specimens of *B. obliquum* (fig. 6, *E, F*) occurs between masses of vascular elements and points of injury.

Of the stelar tissues, the tracheids and sieve tubes are incapable of further growth. The remaining tissues are separated by these into three groups: (1) pith, (2) cambium and adjacent parenchyma, and (3) pericycle. The data given above show that all these three tissues may produce vascular elements, either tracheids or sieve tubes. Hence we may conclude that the production of vascular elements by any stelar tissue is limited only by the capacity of the tissue for further growth.

The manner of that further growth of a stelar tissue varies with the species. In *Ophioglossum pendulum*, which has no secondary thickening, the strands of xylem in the pith of an aberrant specimen were primary in origin. In *Helminthostachys*, no secondary xylem is formed under the usual conditions of development; and renewed growth of the stelar tissues, as in the case of branching, produces an irregular secondary thickening of the stele without a definite meristematic layer. On the other hand, *B. virginianum* and *B. obliquum* show very great development of secondary wood under usual conditions. In both these species, renewed growth of the pith, cambium, and pericycle manifested itself in part by the formation of secondary xylem. Hence we may conclude that the

manner of formation of vascular elements by stelar tissues varies with the species, but is relatively constant in any one species under various conditions.

Examination of the various structures shows that they differ mainly in the tissue producing them and in amount of development. That is, these individual variations are differences of position and quantity of vascular elements, and as such their explanation is to be looked for among the physiological factors operating at the time of their development. From this point of view, vascular structures produced as a result of injury may readily show ancestral characters; but such characters are to be considered, not as the repetition of a definite stage of the phylogenetic development of the form, but rather as an indication of the recurrence of certain conditions of development.

It seems well to insist at this point that vascular strands are secondary structures as compared with the tissues which they traverse. The formation of an organ creates a physiological demand to which the vascular strand is a response; and uniformity of the structure which results is only an indication of uniformity of demand and of uniform conditions of development. In this view, the vascular connections of the branches are determined by factors of the same character as those controlling wound reactions.

The vascular supply must be contributed by stelar tissues capable of growth; these tissues are the ones already enumerated, pith, cambium and adjacent parenchyma, and pericycle, together with the parenchyma between the leaf trace and the leaf gap. The manner of formation of the vascular elements of the branch supply is more or less restricted in any species to the particular method of that species. Thus in *B. ramosum*, in which secondary wood formation is relatively slight, no renewed cambial activity occurred in connection with the formation of a branch; while in *B. obliquum*, in which secondary wood formation is very marked under usual conditions, not only does the cambium begin active growth in every case of branching, but similar cambial activity is sometimes set up in both pith and pericycle. The physiological demand likewise varies; in the branch of *B. ramosum* represented in figs. 4 and 8,

two growing points were doubtless established. This produced a physiological demand which, operating under the conditioning factors just described, produced two branch steles.

The conclusion that is reached, therefore, is that the vascular connections of the branches are determined in general by three factors. These factors are (1) the presence of stelar tissues capable of growth within the range of the influence of the developing branch; (2) the nature of the growth which can be induced in those tissues, in particular, whether such growth is cambial or not; (3) the physiological demand produced by the growing branch. The first of these factors will vary with the distance of the bud from the leaf trace as compared with its distance from the stem stele; and, more particularly, with the age of those structures when the branch begins to develop. The second factor may be considered to be relatively constant for any one species but to vary widely with different species. The vascular connection produced is the direct result of the third factor acting upon and limited by the other two; the individual conditions give to this third factor a special value for each particular case; the resultant structures are therefore direct responses to unknown and varying conditions and of physiological interest only. It is therefore concluded that the vascular connections of the branches of *Botrychium* have little or no phylogenetic significance.

THE ORIGIN AND DEVELOPMENT OF THE AXILLARY BUDS

In view of the foregoing conclusion, attention has been turned from the anatomy of the branch connections to the examination of the origin of the axillary buds. Since LANG'S description of these buds in *B. Lunaria* deals only with the mature structures, the further investigation has been directed toward the examination of the origin and development of these buds in *B. obliquum*.

The apical region.—The apical region of *Botrychium* has been described in detail by HOLLE (6), CAMPBELL (3), and BRUCHMANN (2). All agree that growth takes place by means of an apical cell of the form of a triangular pyramid, and that each segment of the apical cell probably gives rise to a leaf. BRUCHMANN states that in *B. Lunaria* leaf formation begins by the appearance of an apical

cell within a segment; and that the entire segment rises abruptly above the plane of the apex of the stem.

In connection with the investigation of the origin of the axillary buds, the apical region of *B. obliquum* has been examined, and the results will be given briefly. As shown by figs. 13 and 14, the apical cell is a triangular pyramid, with three cutting faces. The first division of a segment is by a periclinal wall, as stated for *B. virginianum* by CAMPBELL; the further divisions are irregular.

While the limits of the various segments cannot always be exactly defined, it seems certain that each segment gives rise to a leaf; hence one segment is cut off each year. Fig. 13 represents a transverse section through the apical region of a plant collected early in April; the first segment was cut off during the preceding year and has divided transversely. In the second year, irregular divisions, both longitudinal and transverse, take place with the segment; at the end of the year, the segment consists of 6-15 cells. During the third year, this irregular division continues; but there is little or no extension above the plane of the apex. At the beginning of the fourth year, the segment begins a much more rapid growth and rises abruptly above the plane of the apex, as described by BRUCHMANN; at about the same time, an apical cell is recognizable within the segment, and the further growth is definitely apical. It is evident that while the entire segment takes part in the initial growth, the leaf is formed from only a part of the segment; the remainder of the segment builds up the stem tissues. By the end of the fourth year, the leaf has become a hemispherical mass which has grown forward and upward, and its forward margin extends considerably beyond the apical cell of the stem (fig. 14). Early in the fifth year, the fertile spike makes its appearance. It is first recognized as an apical cell on the forward side of the apical cell of the leaf; by the end of the year, it has produced a well defined knoblike structure. During the sixth year, both the fertile and sterile portions of the leaf develop rapidly. In the summer of the seventh year, this leaf breaks through the base of the enveloping older leaf, and the spores are shed in September. The fertile spike then withers, but the sterile portion of the leaf persists

through the winter, and only dies in the early summer of the eighth year, after the emergence of the next younger leaf.

The bud.—As stated above, the first evidence of the formation of a new leaf is the abrupt rise of the entire segment above the plane of the apex. This growth is relatively slight in amount, and the further development of the young leaf is apical. The cells produced by the apical growth are arranged in such definite rows (fig. 15) that it is easy to distinguish the tissue produced by the apical cell.

The axillary bud can first be distinguished on the adaxial face of the base of a young leaf, after the apical growth of the leaf has proceeded to a considerable extent (fig. 15). At this time, it consists of a plate of meristematic cells, 6–8 in number; there is no evidence of an apical cell. The arrangement of the surrounding cells shows clearly that the bud has not come from the apical cell of the leaf, but has arisen from cells carried up by the elongation of the segment. By the upgrowth of the apical region of the stem, this plate of meristematic cells is thrown into a crevice, which lies between the stem and the adaxial face of the base of the leaf; fig. 14 shows the appearance of a bud a year older than that shown by fig. 15. It consists of a mass of 30–40 cells, lying as a plate at the base of the leaf; one of the cells shown has divided by a periclinal wall, but such a division occurs rarely.

In older leaves, the location of this crevice or slit is readily pointed out by the margin of the stipular sheath of the next younger leaf; this makes the identification of the buds in longitudinal sections of the rhizome particularly easy. Fig. 16 shows the margin of the stipular sheath of the functioning leaf of a rhizome, and the bud formed in the axil of the leaf of the preceding year; fig. 17 shows a bud a year older. They consist of plates of cells of a meristematic nature; these plates are never more than two cells in thickness and are usually 5–8 cells in longitudinal extent. In tangential sections of a rhizome (figs. 18, 19), they are seen to be 6–10 cells in width; the canal by which they communicate with the exterior is a mere slit. There is not the slightest evidence of any apical cell, or other indication of an apex. They agree in all essentials with the buds of *B. Lunaria* as described by LANG.

Discussion.—As stated above, neither axillary nor adventitious buds occur in the rhizomes of either *Ophioglossum vulgatum* or *O. pendulum*; and where branching occurs in these species, it is dichotomous. On the other hand, the buds and branching of *Helminthostachys* resemble those of *Botrychium* in every respect. These facts may be considered further evidence of the close relationship of these two genera; at the same time, they will serve to emphasize the differences between these two genera on the one hand and *Ophioglossum* on the other.

LANG (9) has mentioned the similarity between the vascular connection of the branches of *Botrychium* and of species of the Hymenophyllaceae. For purposes of comparison, the origin of the axillary buds of a species of *Trichomanes* from Samoa has been examined. The leaf has an apical cell from the beginning and develops to a considerable extent before the branch appears. The formation of the branch is initiated by the appearance of an apical cell. The exact method of formation of this apical cell of the branch was not determined; but it is evident that it arises late and from the growing point of the leaf. The axillary branches of *Trichomanes*, therefore, are foliar in origin. In *Botrychium*, as shown above, the axillary bud is in no way related to the apical cell of the leaf; it arises directly from a portion of a segment of the apical cell of the rhizome; and its position on the base of the leaf is incidental and does not indicate a foliar origin. The similarity between the branching of *Botrychium* and *Trichomanes*, therefore, is not close.

The mature axillary buds of *Botrychium* are of the simplest possible form, an undifferentiated layer of meristematic cells; and it is to be noted that at no time in their development is there any differentiation. This may be accepted as evidence of reduction produced in connection with dormancy; and we may conclude that *Botrychium* and *Helminthostachys* have been derived from a form which branched freely in a monopodial fashion. This is in full agreement with other evidence which points to a relationship of the Ophioglossaceae to the primitive forms of Filicales, especially the Zygopterideae.

Summary

1. Branching of the rhizome of *Ophioglossum vulgatum* and *O. pendulum* is dichotomous; there are no axillary or adventitious buds on the rhizome.
2. Axillary buds are regularly present in five species of *Botrychium*.
3. The vascular connections of the branches in *Botrychium* vary widely with the species and with the individual specimen. It is concluded that the details of the vascular supply of the branch are controlled by the conditions of development and are therefore of little or no phylogenetic importance.
4. In wounded rhizomes of *B. obliquum*, renewed activity of the cambium produces considerable masses of accessory xylem; the pith frequently develops sieve tubes and a cambium which produces secondary xylem in quantity; the pericycle often produces sieve tubes and secondary xylem. It is concluded that in this species any stelar tissues capable of growth may produce vascular elements under the influence of an injury.
5. The axillary bud of *B. obliquum* arises as a plate of meristematic cells on the adaxial face of the base of the very young leaf; it develops without differentiation into a plate of meristematic tissue one or two cells in thickness and 50-60 cells in area, which is buried by overgrowth of surrounding tissue.
6. The data secured is in agreement with the evidence pointing to a relationship of the Ophioglossaceae to the primitive ferns, especially the Zygopterideae.

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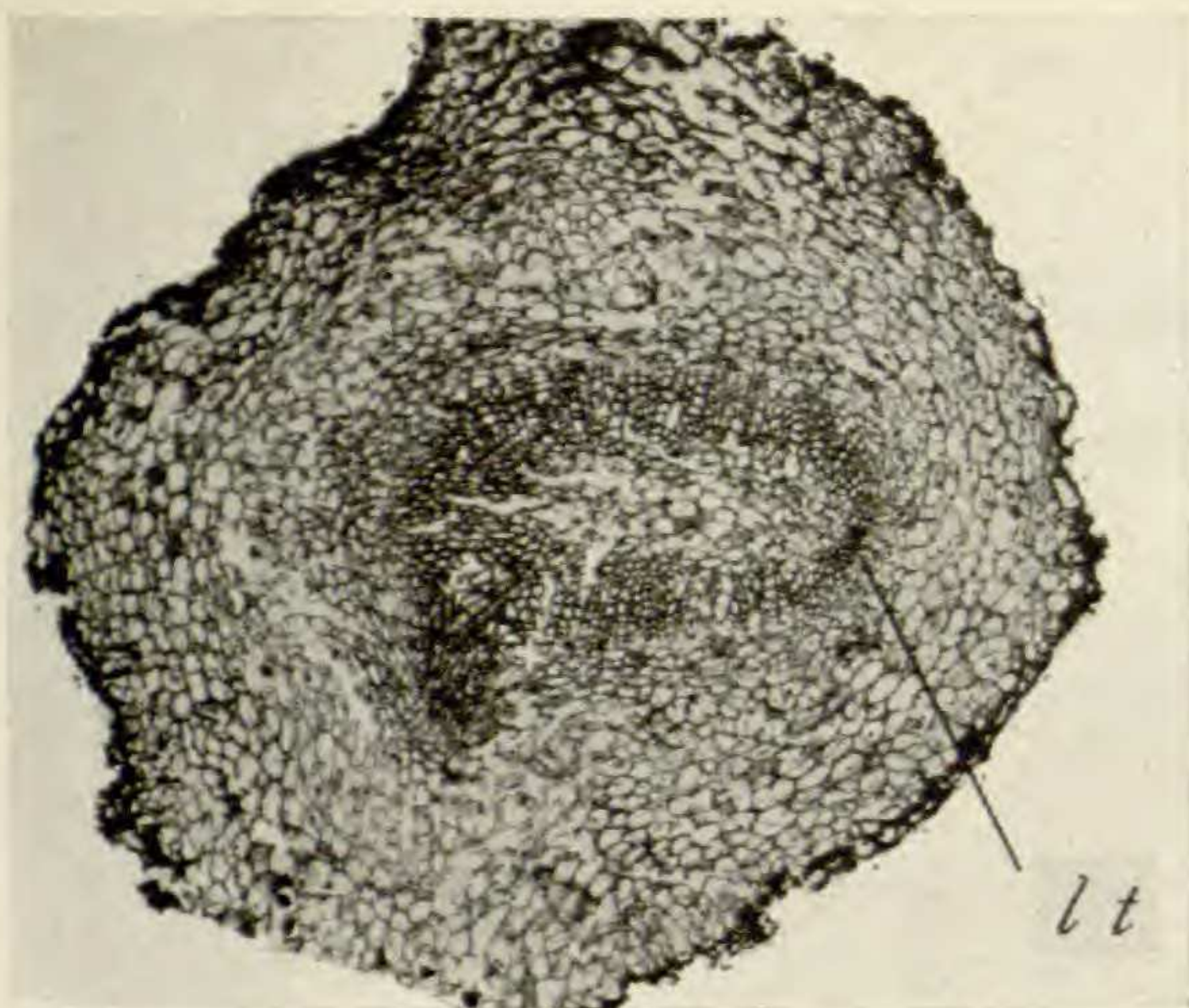
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EXPLANATION OF PLATES XX AND XXI

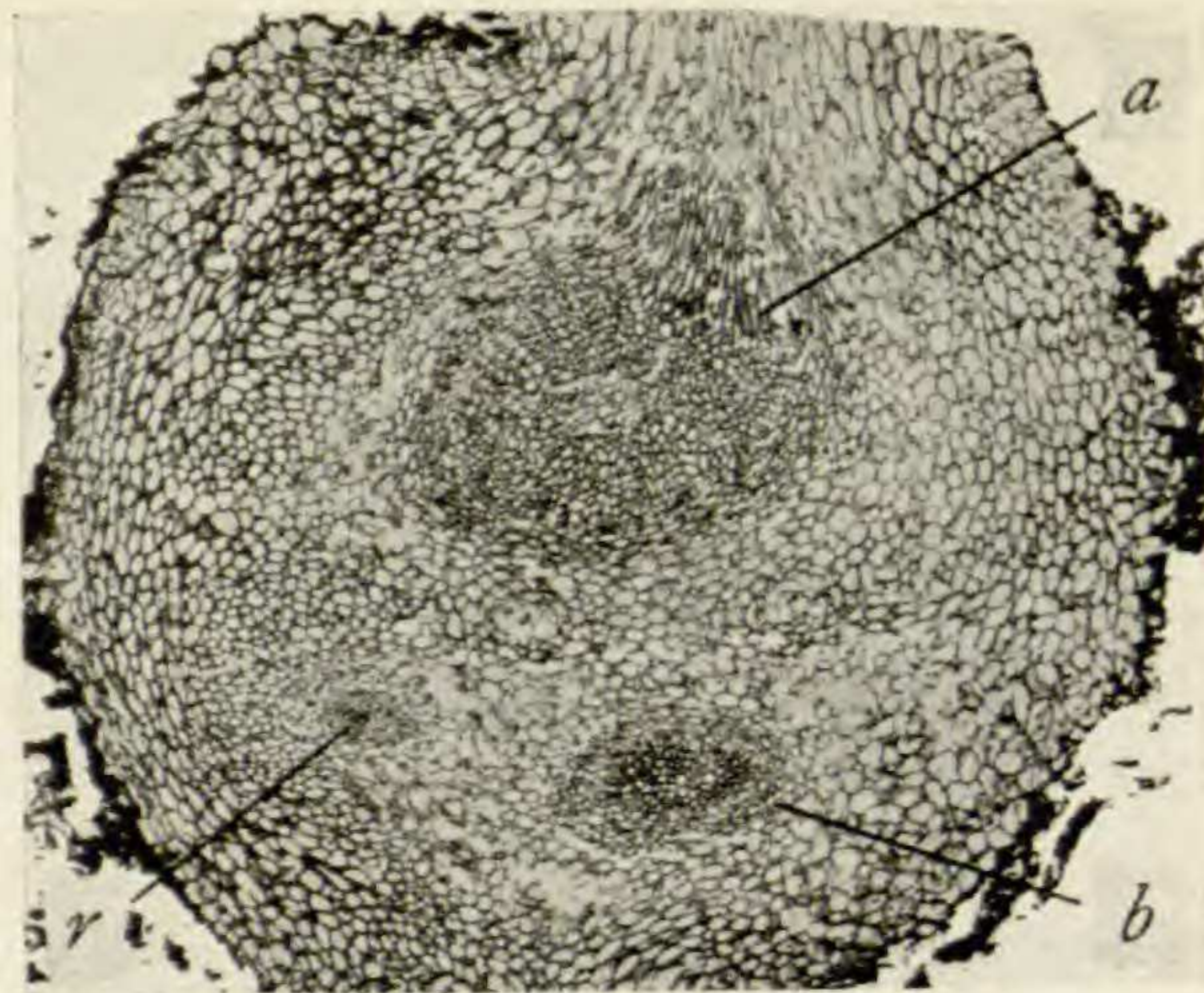
(Figs. 1-6 are in the text)

FIG. 7.—Transverse section of a rhizome of *Botrychium ramosum* at the base of a branch: *lt*, leaf trace; $\times 20$.

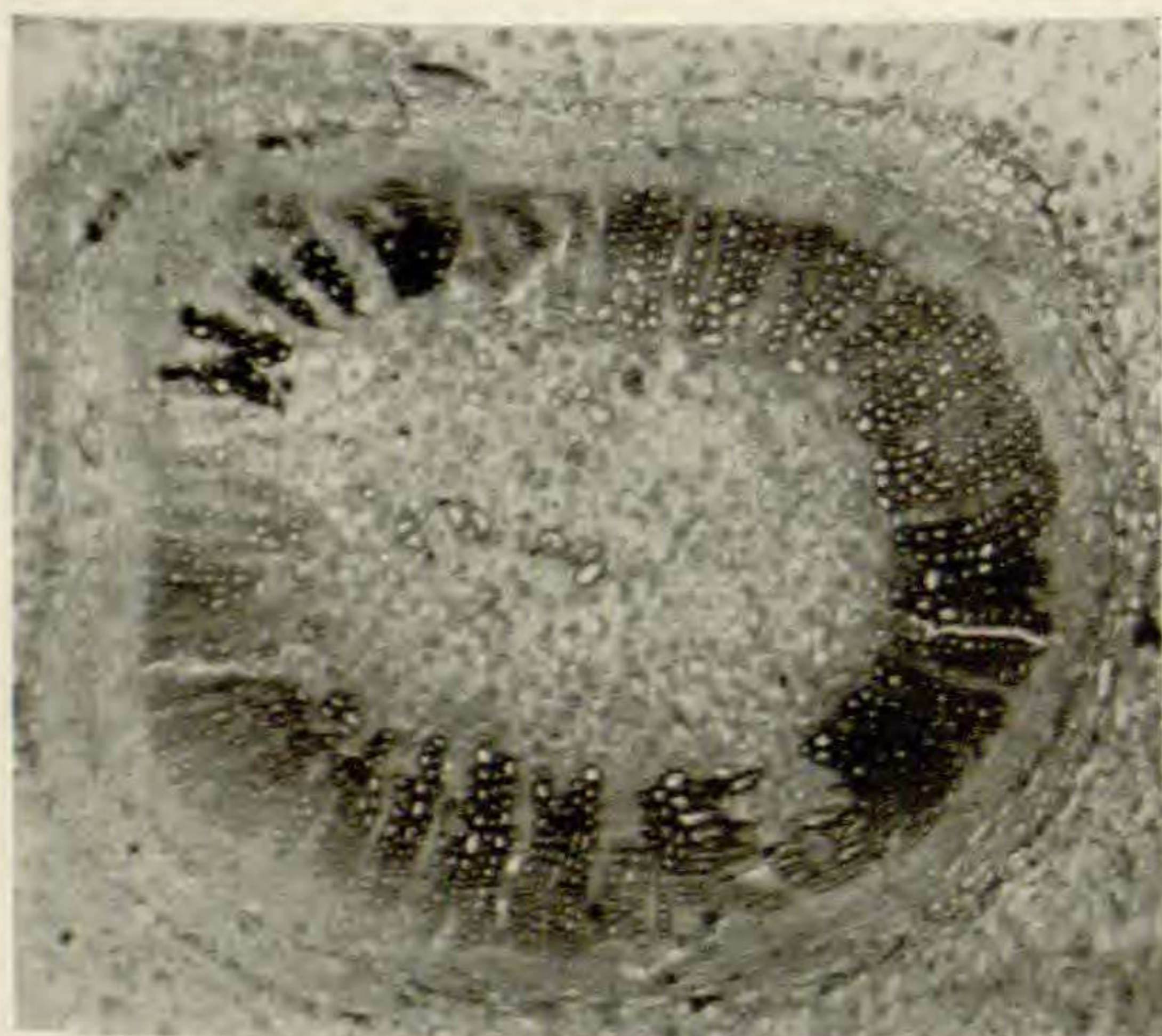
FIG. 8.—Transverse section of the branch whose origin is represented by figs. 4 and 7, showing the two branch steles: *a*, larger stele of the branch; *b*, smaller stele of the branch; *r*, trace of root which connects with the smaller branch stele; $\times 20$.



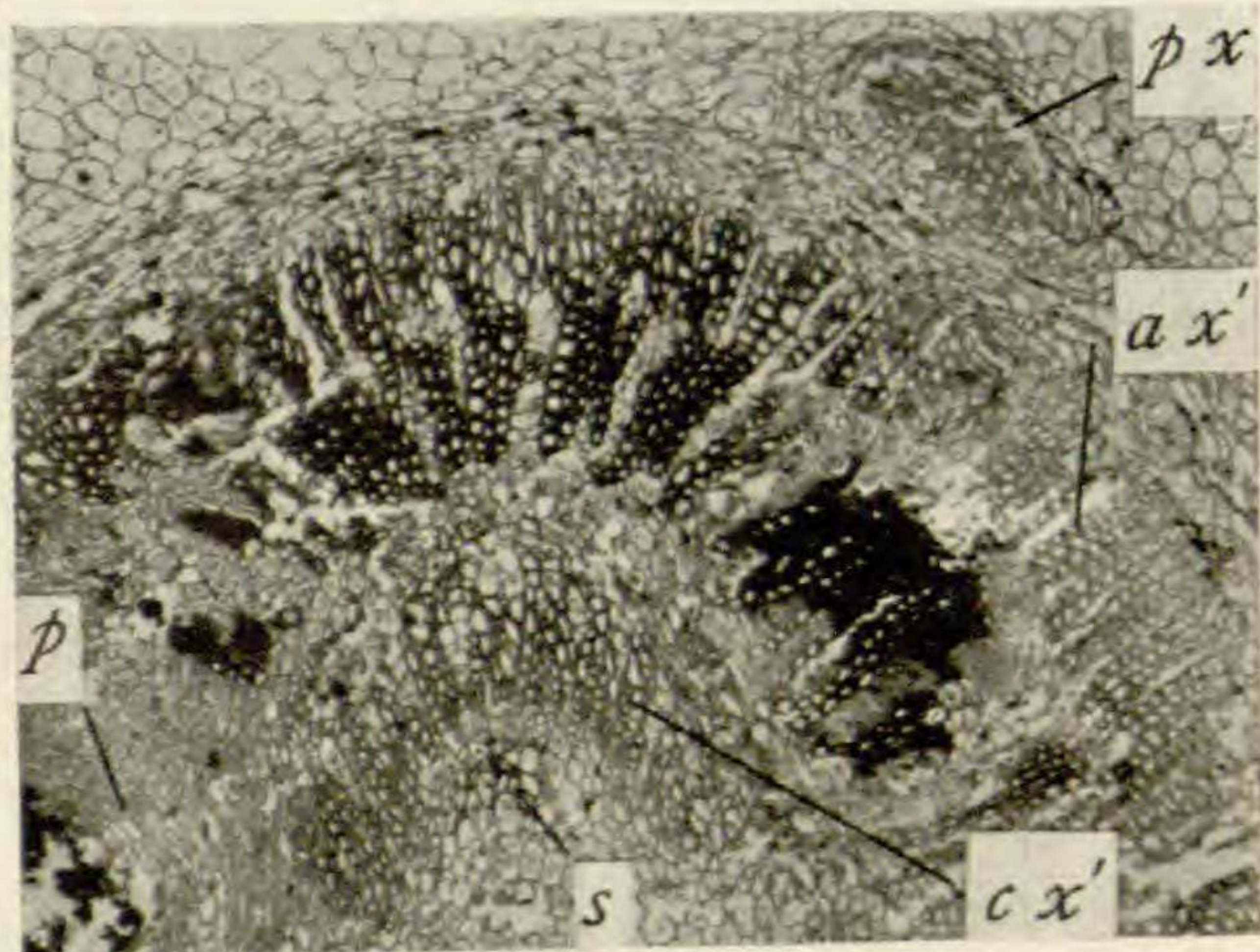
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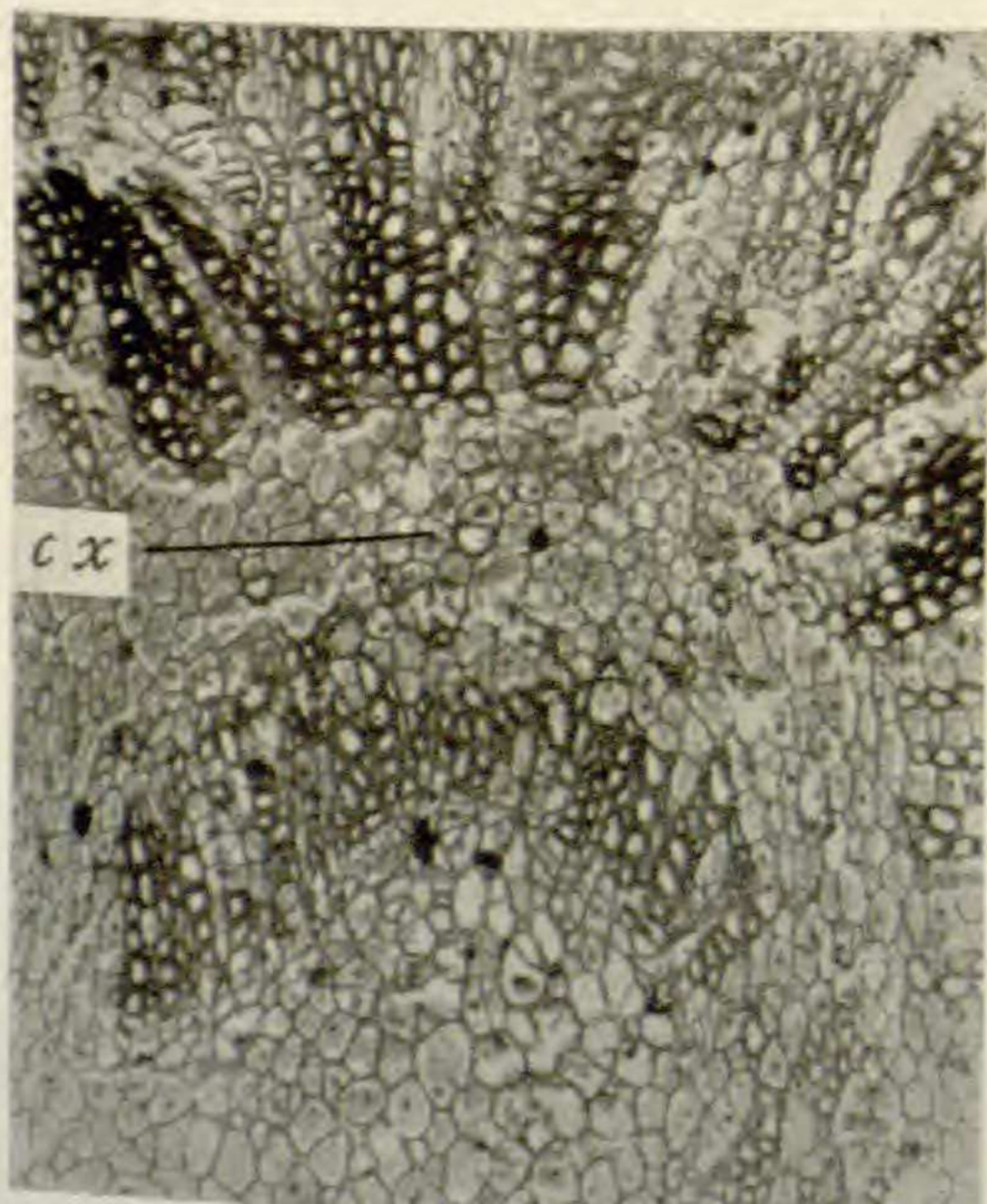
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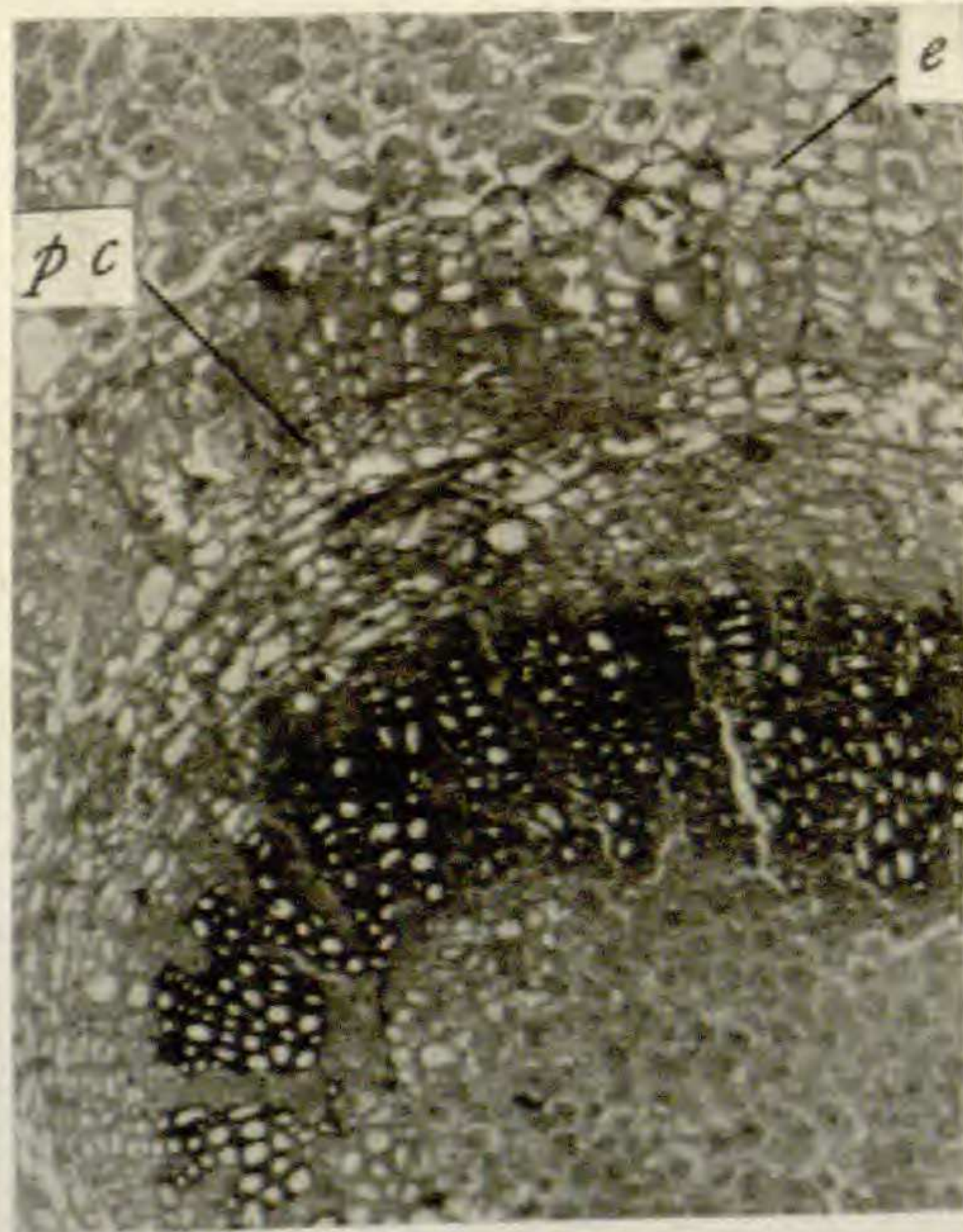
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